# Glucagonostatic Actions and Reduction of Fasting Hyperglycemia by Exogenous Glucagon-Like Peptide I(7–36) Amide in Type I Diabetic Patients

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**OBJECTIVE** — Glucagon-like peptide I(7–36) amide (GLP-I) is a physiological incretin hormone that, in slightly supraphysiological doses, stimulates insulin secretion, lowers glucagon concentrations, and thereby normalizes elevated fasting plasma glucose concentrations in type II diabetic patients. It is not known whether GLP-1 has effects also in fasting type I diabetic patients.

**RESEARCH DESIGN AND METHODS** — In 11 type I diabetic patients ($HbA_{1c}$ 9.1 ± 2.1%; normal, 4.2–6.3%), fasting hyperglycemia was provoked by halving their usual evening NPH insulin dose. In random order on two occasions, 1.2 pmol · $kg^{-1}$ · $min^{-1}$ GLP-I or placebo was infused intravenously in the morning (plasma glucose 13.7 ± 0.9 mmol/l; plasma insulin 26 ± 4 pmol/l). Glucose (glucose oxidase method), insulin, C-peptide, glucagon, GLP-I, cortisol, growth hormone (immunoassays), triglycerides, cholesterol, and nonesterified fatty acids (enzymatic tests) were measured.

**RESULTS** — Glucagon was reduced from ~8 to 4 pmol/l, and plasma glucose was lowered from 13.4 ± 1.0 to 10.0 ± 1.2 mmol/l with GLP-I administration (plasma concentrations ~100 pmol, $P < 0.0001$), but not with placebo (14.2 ± 0.7 to 13.2 ± 1.0). Transiently, C-peptide was stimulated from basal 0.09 ± 0.02 to 0.19 ± 0.06 nmol/l by GLP-I ($P < 0.0001$), but not by placebo (0.07 ± 0.02 to 0.07 ± 0.02). There was no significant effect on nonesterified fatty acids ($P = 0.34$), triglycerides ($P = 0.57$), cholesterol ($P = 0.64$), cortisol ($P = 0.40$), or growth hormone ($P = 0.53$).

**CONCLUSIONS** — Therefore, exogenous GLP-I is able to lower fasting glycemia also in type I diabetic patients, mainly by reducing glucagon concentrations. However, this alone is not sufficient to normalize fasting plasma glucose concentrations, as was previously observed in type II diabetic patients, in whom insulin secretion (C-peptide response) was stimulated 20-fold.

Glucagon-like peptide I(7–36) amide (GLP-I) is an insulinotropic hormone secreted from enteroglucagon-producing cells in the lower gut, i.e., the ileum and colon/rectum (1). Because plasma concentrations increase after meals and reach the concentration range that is necessary to stimulate insulin secretion, GLP-I, together with gastric inhibitory polypeptide from the upper gut, act as physiological incretin hormones (2,3). In concentrations that exceeded postprandial values by a factor of 3–4, exogenous GLP-I [(7–36) or (7–37) amide] raised insulin and lowered glucagon concentrations also in type II diabetic patients (4,5). It also reduced meal-related insulin requirements in both type I and type II diabetic patients (6). However, after a mixed meal, not only influences on insulin and glucagon secretion but also other effects, such as an inhibition of gastric emptying (7,8), have probably contributed to the antidiabetogenic effect (6). Effects of exogenous GLP-I in fasting type I diabetic patients have not been studied so far. Therefore, the aim of the present study was to characterize the endocrine pancreatic and glucose response to a pharmacological dose of exogenous GLP-I in fasting type I diabetic patients. Preliminary results have been communicated in abstract form (9).

## RESEARCH DESIGN AND METHODS

### Study protocol

The study protocol was approved by the ethics committee of the medical faculty of the Georg-August-University in Göttingen, Germany, 16 December 1992. Written informed consent was obtained from all participants.

### Patients

Eleven type I diabetic patients were studied (Table 1). Those patients who were most likely to still have a normal glucagon secretory capacity (which is partially lost in type I diabetes of long duration [10,11]) but not able to secrete considerable amounts of C-peptide (endogenous insulin) were selected. So all patients were well beyond their honeymoon period, and the duration of diabetes was 7 ± 3 (SD) years. They were all being treated with insulin, most of them with an intensive treatment regimen that led to satisfactory metabolic control ($HbA_{1c}$ 9.1 ± 2.1%; Table 1). Patients with severe retinopathy, neuropathy, or nephropathy were excluded. At the time of the study, the patients were in good general health. Transaminases were normal, as were

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ELISA, enzyme-linked immunosorbent assay; GLP-I, glucagon-like peptide I(7–36) amide; IR, immunoreactive; NEFA, nonesterified fatty acid; RM-ANOVA, repeated measurement analysis of variance.

Table 1—*Patient characteristics*

| | Sex (M/F) | Age (years) | BMI (kg/m$^2$) | Duration of diabetes (years) | C-peptide (nmol/l) | Insulin dose (U/kg) | Islet cell antibody at diagnosis |
|---|---|---|---|---|---|---|---|
| Patient number | | | | | | | |
| 1 | M | 26 | 25.3 | 1 | 0.15 | 0.6 | Positive |
| 2 | M | 29 | 25.7 | 8 | 0.10 | 1.1 | Positive |
| 3 | M | 30 | 22.4 | 10 | 0.03* | 0.6 | NA |
| 4 | M | 31 | 22.2 | 14 | 0.03* | 0.7 | Positive |
| 5 | M | 25 | 20.5 | 5 | 0.03* | 0.7 | NA |
| 6 | M | 25 | 29.5 | 3 | 0.18 | 0.6 | Positive |
| 7 | M | 34 | 22.3 | 8 | 0.06 | 0.6 | Positive |
| 8 | M | 34 | 21.6 | 7 | 0.10 | 0.6 | Positive |
| 9 | M | 32 | 22.9 | 5 | 0.08 | 0.6 | Negative |
| 10 | M | 21 | 21.4 | 9 | 0.08 | 1.0 | NA |
| 11 | F | 26 | 20.5 | 5 | 0.14 | 0.5 | Positive |
| Mean | 10 M | 29 | 23.2 | 7 | 0.09 | 0.7 | 7 positive |
| SD | 1 F | 4 | 2.8 | 3 | 0.05 | 0.2 | 1 negative |

*Patients considered C-peptide–negative. Values of positive islet cell antibody at diagnosis were >20 Juvenile Diabetes Foundation units; GAD II antibodies were positive in patients 1, 4, and 5 at the time of this study (and negative in the remaining patients). NA, not available.

blood cell counts and creatinine plasma values and clearance.

Patients were studied on two occasions in random order. A regular meal and drug schedule was allowed for 1 day between the experiments with GLP-I and placebo. On the study days, all medication (L-thyroxine in two patients) was withheld until the end of the experiments.

## Peptides

Synthetic GLP-I was purchased (Saxon Biochemicals GmbH, Hannover, Germany). The lot number used was PGAS 242, Lot ZE 865 (net peptide content, 79.3%) (3,4). The peptide was dissolved, filtered through 0.2 μm nitrocellulose filters (Millipore, Bedford, Massachusetts), and stored frozen at −30°C as previously described. Net peptide content rather than gross weight was used for dose calculations. High-performance liquid chromatography profiles (provided by the manufacturer) showed that the preparation was >99% pure (single peak coeluting with appropriate standards). Samples were analyzed for bacterial growth (standard culture techniques) and pyrogens (Limulus amoebocyte lysate endo-LAL, Chromogenix AB, Mölndal, Sweden). No bacterial contamination was detected. Endotoxin concentrations in the GLP-I stem solutions always were <0.03 EU/ml.

## Experimental procedures

The tests were performed in the morning after an overnight fast. On the evening before the studies, regular insulin was administered before dinner as usual, but the late-night dose of NPH insulin (usually administered at 10:00 P.M.) was halved to produce insulinopenia and slight hyperglycemia during the experimental period. On the following morning, when no regular insulin was given until the experiment was finished, two forearm veins were punctured with teflon cannula (Moskito 123, 18 gauge, Vygon, Aachen, Germany) and kept patent using 0.9% NaCl (for blood sampling and GLP-I/placebo administration).

After drawing basal blood specimens, at 0 min, an intravenous infusion of GLP-I or placebo (0.9% NaCl containing 1% human serum albumin; Merieux, Norderstedt, Germany) was started at an infusion rate of 1.2 pmol · kg$^{-1}$ · min$^{-1}$ and continued for 240 min. Blood was drawn at 30-min intervals and plasma glucose was determined immediately. The experiments were performed in randomized order.

## Blood specimens

Blood was drawn into heparinized tubes (immunoreactive [IR] insulin and C-peptide measurements). A sample was stored in NaF (Microvette CB 300, Sarstedt, Nümbrecht, Germany) for the measurement of glucose. For glucagon and GLP-I measurements, blood was drawn into tubes containing EDTA and aprotinin (Trasylol; 20,000 KIU/ml, 200 μl per 10 ml blood; Bayer AG, Leverkusen, Germany). After centrifugation, plasma for hormone analyses was kept frozen at −30°C.

## Laboratory determinations

Glucose was measured in plasma using a glucose oxidase method with a Glucose Analyzer 2 (Beckman, Munich, Germany). Plasma IR insulin (IMx insulin, Abbott, Wiesbaden, Germany) and C-peptide (enzyme-linked immunosorbent assay [ELISA], DRG, Marburg, Germany) were determined using commercial immunoassay kits, with human insulin and C-peptide as standard. The working range for the insulin assay was 6–1,300 pmol/l. There was ~0.005% cross-reactivity with proinsulin or conversion intermediates. The intra-assay coefficient of variation was 2.5–4.0%, and the inter-assay coefficient of variation was 3.4–4.5%. The working range for the C-peptide assay was 0.02–5.0 nmol/l. There was ~0.5% cross-reactivity with proinsulin or conversion intermediates. The intra-assay coefficient of variation was 3.2–8.2%, and the interassay coefficient of variation was 7.3–9.8%.

Insulin antibodies were excluded in all patients using SynELISA Insulin-Antikörper (cholesterol oxidase–phenol 4 aminophenazone peroxidase; ELIAS, Freiburg, Germany) (all values <3.7 U/ml, normal <12). GAD-II antibodies were determined using SynELISA GAD-II-Antikörper (ELIAS).

IR GLP-I was determined in etha-

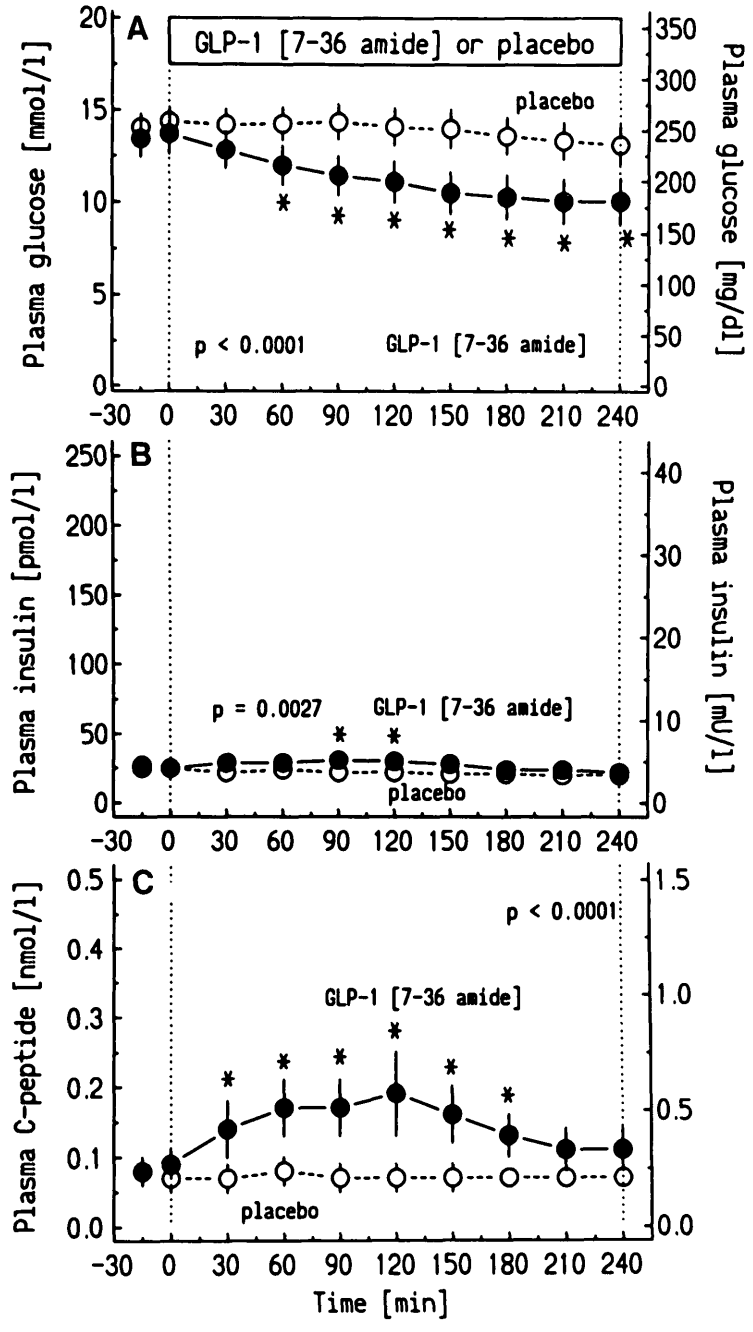


**Figure 1**—*Plasma glucose (A), insulin (B), and C-peptide (C) responses to the intravenous administration of GLP-I (1.2 pmol · kg$^{-1}$ · min$^{-1}$) or placebo in 11 type I diabetic patients (mean ± SE). ●, experiments with GLP-I; ○, experiments with placebo. The bar indicates the duration of exogenous administration of GLP-I or placebo. P values are derived by RM-ANOVA (indicating significant interactions of treatment and time). Asterisks indicate significant differences at single time points (paired t tests, P < 0.05).*

nol-extracted plasma as previously described (13), using antiserum 89390 (final dilution, 1:150,000) and synthetic GLP-1 for tracer preparation and as standard. Recovery of GLP-1 standards after alcohol extraction was 75 ± 8%. The experimental detection limit (2 SD over samples not containing GLP-I) was <5 pmol/l. Antiserum 89390 binds exclusively to proglucagon-derived peptides containing the amidated COOH-terminal GLP-1 sequence. The intra-assay coefficient of variation was 6%, and the inter-assay coefficient of variation was 11%. Pancreatic glucagon was assayed in ethanol-extracted plasma using antibody 4305 (14). Nonesterified fatty acids were quantitated enzymatically with acyl-CoA oxidase from *Candida tropicalis* using a Hitachi 705 autoanalyzer. Reagents were from Wako Chemicals (Neuss, Germany).

Triglycerides (Peridochrom Triglycerid, glycerol 3 phosphate oxidase–phenol 4 aminophenazone peroxidase) and cholesterol (Monotest Cholesterin, cholesterol oxidase–phenol 4 aminophenazone peroxidase) were also measured on a Hitachi 705 autoanalyzer using reagents from Boehringer Mannheim (Mannheim, Germany).

Cortisol was determined using a solid phase radioimmunoassay (Diagnostic) obtained from Hermann Biermann Diagnostika (Bad Nauheim, Germany).

Growth hormone was assayed using a double-antibody radioimmunoassay (Diagnostic) obtained from Hermann Biermann Diagnostika. Each patient's set of plasma samples was assayed at the same time to avoid errors due to interassay variation.

### Statistical analysis

Results are reported as mean ± SE. Integration was carried out according to the trapezoidal rule. Significances of differences were tested using repeated measurement analysis of variance (RM-ANOVA; NCSS version 5.01, Kaysville, UT). If a significant interaction of treatment and time was documented ($P < 0.05$), values at single time points were compared by Student's *t* test (paired analyses). A corrected two-sided *P* value <0.05 was taken to indicate significant differences.

## RESULTS

### Glucose

Halving the evening NPH insulin dose produced moderate fasting hyperglycemia (13.7 ± 0.8 mmol/l) and relatively low circulating insulin concentrations (26 ± 4 pmol/l; normal values in fasting healthy subjects were ~30–75 pmol/l when using the same assay methodology). With placebo infusion, insulin concentrations further declined during the 4 h of the experiments, and plasma glucose concentrations did not change considerably (0 min: 14.4 ± 0.8 mmol/l; 240 min: 13.1 ± 1.0 mmol/l). With exogenous GLP-1, glycemia was reduced from 13.7± 1.0 mmol/l (0 min) to 10.0 ± 1.2 mmol/l (240 min; $P < 0.0001$; Fig. 1).

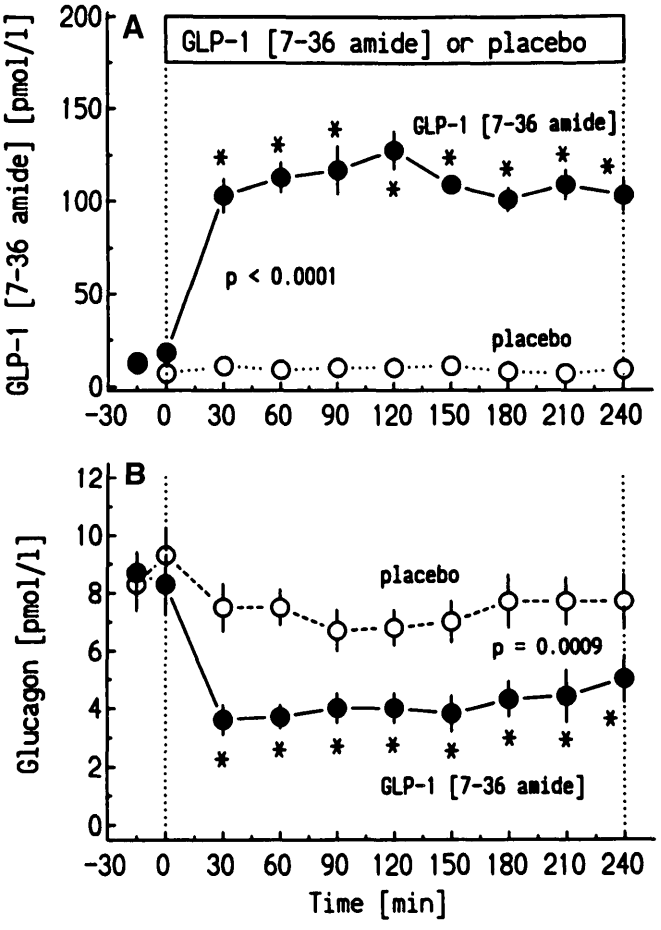


**Figure 2**—*Plasma GLP-1 (A; antibody 89390) and pancreatic glucagon (B; antibody 4305) responses to the intravenous administration of GLP-1 (1.2 pmol · kg$^{-1}$ · min$^{-1}$) or placebo in 11 type I diabetic patients (mean ± SE). ●, experiments with GLP-1; ○, experiments with placebo. The bar indicates the duration of exogenous administration of GLP-1 or placebo. P values are derived by RM-ANOVA (indicating significant interactions of treatment and time). Asterisks indicate significant differences at single time points (paired t tests, P < 0.05).*

### Insulin and C-peptide

In 3 of the 11 type I diabetic patients studied, basal plasma C-peptide was below the detection limit (i.e., <0.05 nmol/l). However, in the remaining patients, measurable C-peptide concentrations were detected. The overall concentration was 0.08 ± 0.02 nmol/l. This value was maintained throughout the placebo experiment. With GLP-1, however, there was a small transient but significant ($P$ < 0.0001) increment in plasma C-peptide concentrations (from 0.09 ± 0.02 to 0.19 ± 0.06 nmol/l; Fig. 1*C*). At the same time, insulin concentrations increased significantly with GLP-1 relative to placebo (Fig. 1*B*).

### GLP-1

Plasma concentrations of GLP-1 were ~10 pmol/l in fasting type I diabetic patients. Exogenous administration of GLP-1 raised plasma concentrations to steady-state levels of ~100 pmol/l (Fig. 2*A*; $P$ < 0.0001).

### Glucagon

Pancreatic glucagon concentrations were immediately suppressed during the exogenous administration of GLP-1 (Fig. 2*B*) and remained at ~50% of basal values for the remainder of the experiment. Placebo did not change glucagon concentrations. The difference was significant ($P$ < 0.0001).

### Other substrates and counterregulatory hormones

There was no significant effect of exogenous GLP-1 versus placebo on plasma free fatty acids (nonesterified fatty acid [NEFA]; $P$ = 0.34; Fig. 3). Neither triglycerides ($P$ = 0.57) nor cholesterol ($P$= 0.64) concentrations were significantly changed by GLP-1. Neither cortisol ($P$ = 0.40) nor growth hormone ($P$ = 0.53) concentrations were influenced by the administration of GLP-1 (data not shown).

### Determinants of GLP-1 effects in type I diabetic patients

The glycemic response to GLP-1 was rather uniform (Fig. 4). The three C-peptide–negative patients (Fig. 4; open symbols) tended to have higher fasting glucose concentrations but responded similarly to those with significant C-peptide response (Fig. 4, closed symbols). The fall in blood glucose response to GLP-1 in the three C-peptide–negative patients amounted to −538 ± 80 mmol · l$^{-1}$ · min and in the eight patients with a small residual C-peptide response to −562 ± 67 mmol · l$^{-1}$ · min ($P$ = 0.85 by Student's $t$ test), which is equivalent to a fall by 3.3 ± 0.6 vs. 3.6 ± 1.0 mmol/l ($P$ = 0.80) between baseline and 240 min, respectively.

**CONCLUSIONS** — The results of the present study clearly show that exogenous GLP-1 also lowers plasma glucose in fasting type I diabetic patients. In such patients, a pharmacological dose of GLP-1, resulting in approximately three- to fourfold higher plasma levels than are observed after meal ingestion (1–3), lowered pancreatic glucagon concentrations and stimulated residual insulin secretion.

As expected for type I diabetic patients, the stimulation of insulin secretion, although statistically significant, was only marginal. However, 3 of the 11 patients examined were C-peptide negative (C-peptide, <0.05 nmol/l) in the basal state and demonstrated no clear increment under the influence of GLP-1. Nevertheless, the few residual B-cells that may be present in the remaining type I diabetic patients seem to be responsive to GLP-1. Whether or not this small insulin secretory response contributed to the effect on plasma glucose cannot be clearly determined on the basis of the present data.

The suppression of pancreatic glucagon probably was the major mechanism leading to a fall in plasma glucose in this group of type I diabetic patients. This effect was highly significant. An immediate and sustained response was observed, with a tendency of glucagon values to rise during the ongoing GLP-1 infusion, probably reflecting the fall in plasma glucose.

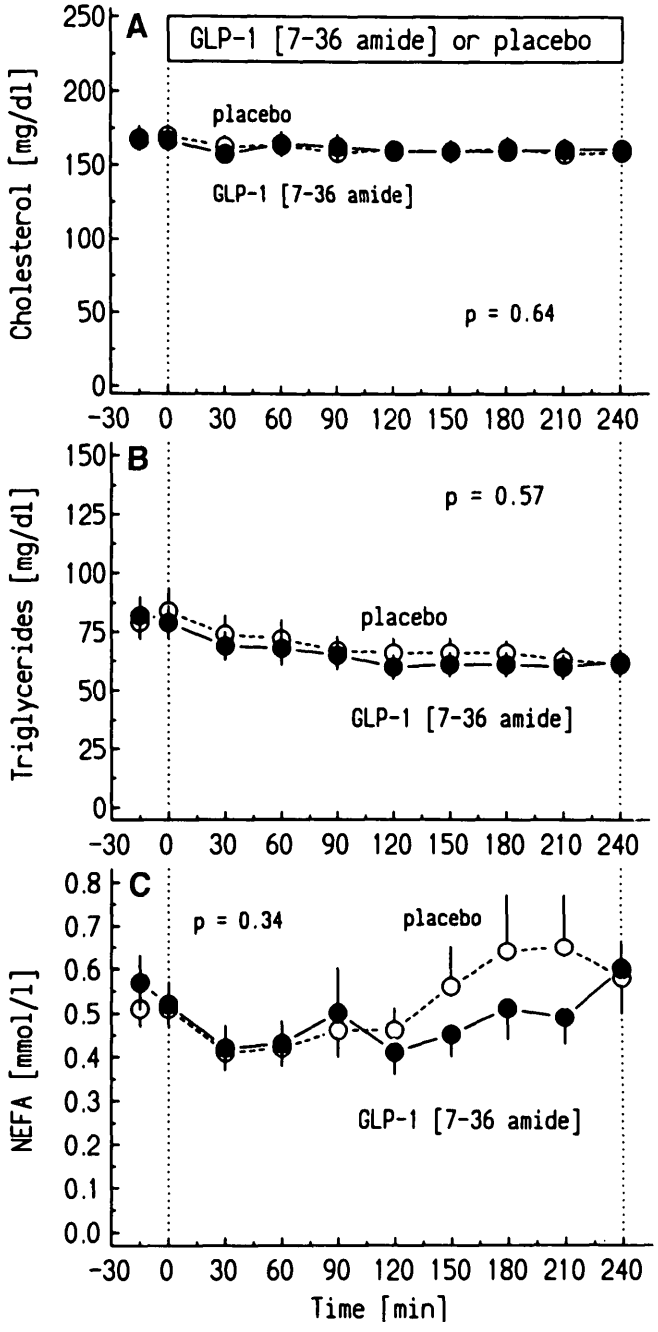


**Figure 3**—*Plasma triglycerides (A), cholesterol (B), and NEFA (C) responses to the intravenous administration of GLP-1 (1.2 pmol · kg$^{-1}$ · min$^{-1}$) or placebo in 11 type I diabetic patients (mean ± SE). ●, experiments with GLP-1; ○, experiments with placebo. The bar indicates the duration of exogenous administration of GLP-1 or placebo. P values are derived by RM-ANOVA (indicating significant interactions of treatment and time).*

It is interesting to compare the present results with those previously described in type II diabetic patients (15) (Table 2). Glucose responses starting from similar baseline values were more pronounced in type II diabetic patients, and normalization of plasma glucose was observed in only two type I diabetic patients. Interestingly, also in type I diabetic patients, the lowest single plasma glucose concentration recorded was 3.6 mmol/l (65 mg/dl), and there was no indication that GLP-I was able to produce hypoglycemia. Insulin and C-peptide responses were 20-fold greater in type II diabetic patients than in the type I diabetic patients from the present study. The fall in glucagon was comparable, irrespective of the type of diabetes (Table 2). This suggests that this GLP-I effect is independent from the presence of functioning B-cells.

Free fatty acids were clearly suppressed by GLP-I in type II diabetic patients but not in the presently studied group of type I diabetic patients (Table 2). This may point to the importance of a significant insulin response for the suppression of lipolysis and to differences in the metabolic states between type I and type II diabetic patients in general. This could mean that the fatty acid–lowering activity of GLP-I in type II diabetic patients (15) was largely mediated by changes in insulin and that in type I diabetic patients, no beneficial effects can be expected.

The effect of GLP-I [(7–36) or (7–37) amide] has not yet been investigated in fasting type I diabetic patients. It had been shown previously that GLP-I reduced meal-related insulin requirements in type I diabetic patients (6), but this could have been secondary to the inhibition of gastric emptying (7,8). However, in the same study, during hyperinsulinemic-euglycemic clamp experiments, glucose infusion rates necessary to counteract the effects of steady-state insulin concentrations were higher with exogenous GLP-I (6). It was not reported whether there were differences in glucagon concentrations between clamp experiments with placebo and GLP-I (6). In another recent examination, the increment in glycemia after a meal was reduced for the duration of exogenous administration of GLP-I in type I diabetic patients, with a rebound after stopping the infusion (16). This also could be related to an inhibitory effect on gastric emptying, which fades when plasma GLP-I concentrations decline after stopping its administration. Furthermore, this group of type I diabetic patients was specifically selected to have a significant residual β-cell function because of immunosuppressive treatment after the diagnosis of type I diabetes and a short diabetes duration.

The question may be asked whether the suppression of glucagon alone or in conjunction with the almost negligible stimulation of residual insulin secretion can explain the fall in glycemia observed in the present study in type I diabetic patients. It appears plausible that the glucagon-lowering effect is of prime importance. The impact of the almost

**Table 2**—*Comparison of integrated responses of glucose, insulin, C-peptide, glucagon, and NEFAs in response to exogenous GLP-1 or placebo in fasting type II and type I diabetic patients*

| Integrated response | Type II diabetic patients | | | Type I diabetic patients | | | Difference type II vs. type I |
|---|---|---|---|---|---|---|---|
| | GLP-1 | Placebo | *P* value | GLP-1 | Placebo | *P* value | |
| Glucose (decrease) (mmol · $l^{-1}$ · min) | 1,097 ± 87 | 347 ± 37 | 0.0005 | 556 ± 52 | 182 ± 43 | 0.0005 | <0.0001 |
| Insulin (increase) (nmol · $l^{-1}$ · min) | 17.4 ± 4.7 | 0.1 ± 0.1 | <0.0001 | 0.9 ± 0.4 | 0.1 ± 0.1 | 0.041 | 0.007 |
| C-peptide (increase) (nmol · $l^{-1}$ · min) | 228 ± 39 | 3 ± 1 | 0.0002 | 15 ± 6 | 1 ± 1 | 0.023 | 0.0004 |
| Glucagon (decrease) (pmol · $l^{-1}$ · min) | 1,418 ± 308 | 510 ± 100 | 0.0097 | 1,014 ± 151 | 420 ± 123 | 0.0027 | 0.24 |
| NEFA (decrease) (mmol · $l^{-1}$ · min) | 26.3 ± 3.1 | 6.7 ± 2.0 | 0.0004 | 24.6 ± 4.3 | 16.4 ± 3.6 | 0.21 | 0.75 |

Data are means ± SE. *P* values were derived using paired or unpaired *t* tests, as appropriate. Data for patients with type II diabetes are taken from Nauck et al. (15), and data for patients with type I diabetes are from this study. Data on differences are from experiments with GLP-1 infusion. Data for glucose are direction over/below baseline values. $n = 10$ for type II patients; $n = 11$ for type I patients.

negligible insulinotropic effect is more difficult to estimate. Also, there are no data to estimate the contribution of possible extrapancreatic effects of GLP-1. Such effects have been described in vitro (17–19), and a change in glucose effectiveness during studies using the minimal model also suggests such actions (20) but could not be confirmed in a recent study (21). Two important questions would be whether possible extrapancreatic effects of GLP-1 occur in vivo and whether they are glucose dependent like those on insulin (2,3,12) and glucagon (22,23) secretion. Otherwise, the stimulation of these pathways could lead to hypoglycemia when GLP-1 is administered for therapeutic purposes, as has been suggested for type II diabetic patients (4–6,15).

The present study raises the question of whether GLP-1 could be an adjunct for the therapy also in type 1-diabetic patients. The comparison to effects in type II diabetic patients demonstrates that the same degree of normalization of fasting glycemia cannot be reached in hyperglycemic type 1 diabetic patients, as has previously been observed in type II diabetic patients (15). Because the main mechanism in type I diabetic patients seems to be the reduction in circulating glucagon, it may be considered to suppress glucagon in those patients whose satisfactory metabolic control is hampered by uncontrolled glucagon secretion (24,25), as has been described in some "brittle" diabetic patients (26).

In conclusion, exogenous GLP-1 in a pharmacological dose reduces fasting glycemia not only in type II diabetic patients, but also in type I diabetic patients. The major mechanism appears to be the glucagon-lowering effect of GLP-1. This finding could be a rational basis for the use of GLP-1 as an adjunct to insulin therapy in type I diabetic patients.

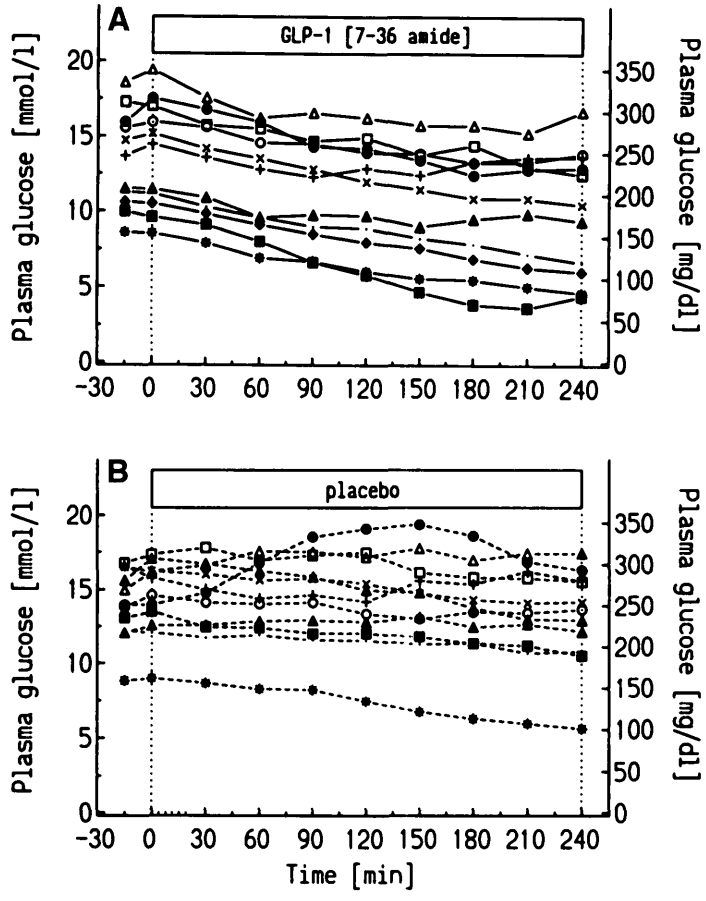


**Figure 4**—*Individual plasma glucose concentrations in fasting type I diabetic patients during the intravenous infusion of GLP-1 (1.2 pmol · $kg^{-1}$ · $min^{-1}$; A) and placebo (B). C-peptide–negative patients (fasting value on two occasions <0.05 nmol/l, no response to GLP-1 administration) are shown with open symbols; others are shown with closed symbols.*

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